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Title: Agnostic Immunity

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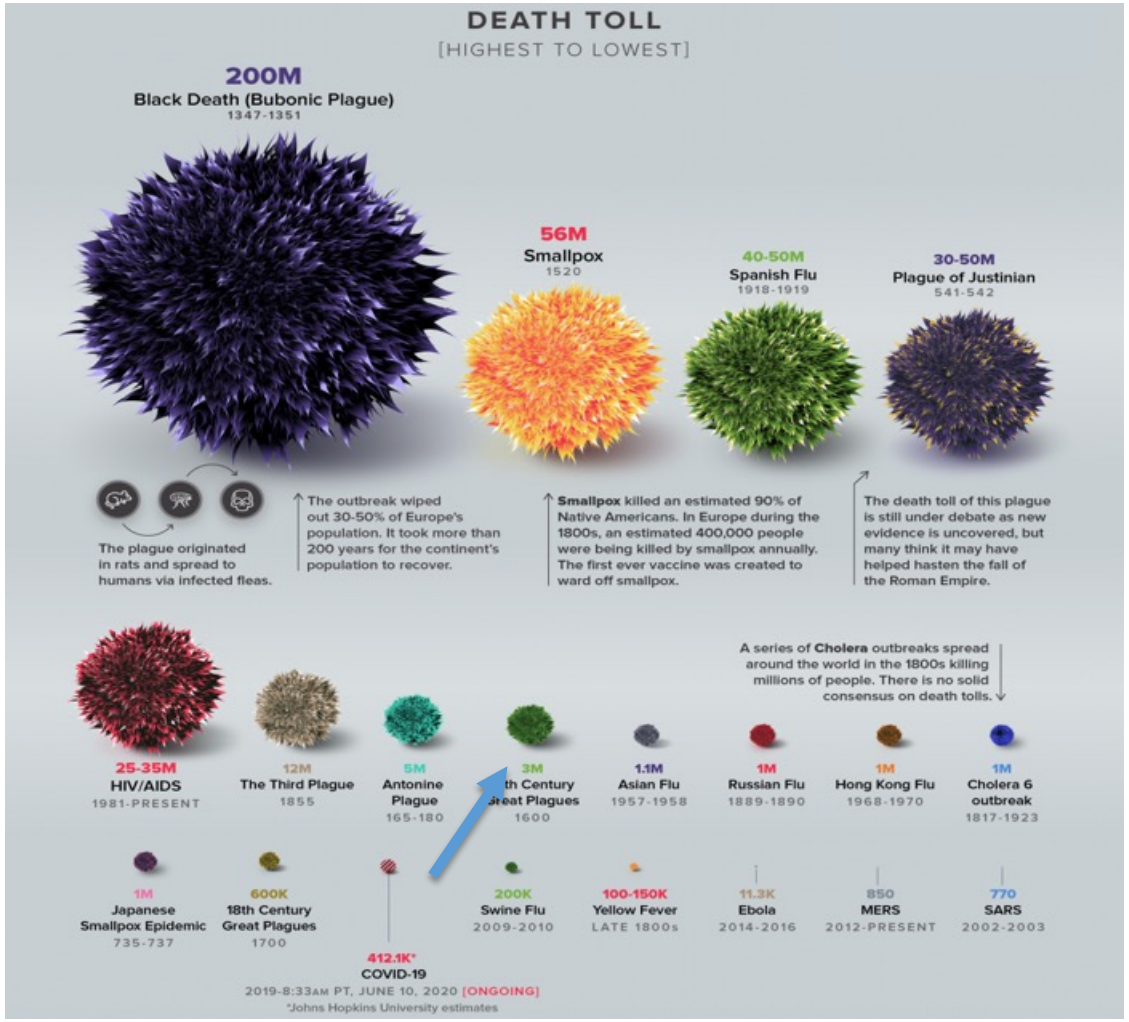
Los Alamos NATIONAL LABORATORY

Agnostic Immunity

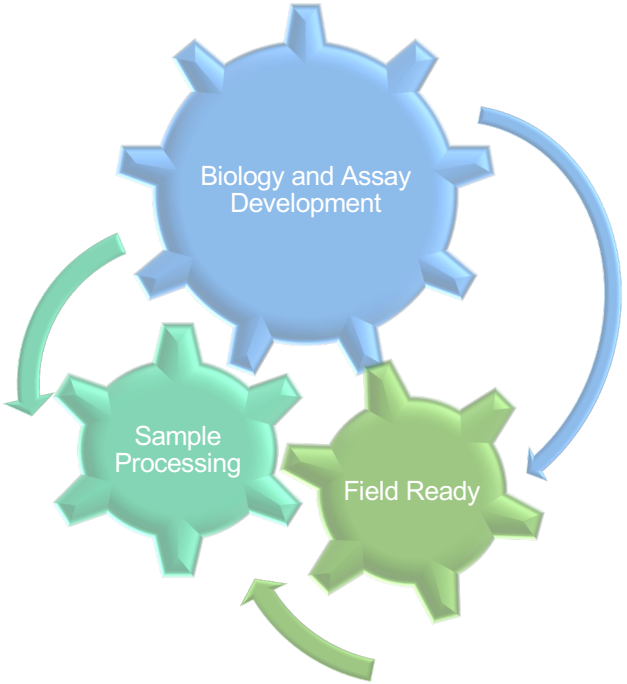
Harshini Mukundan

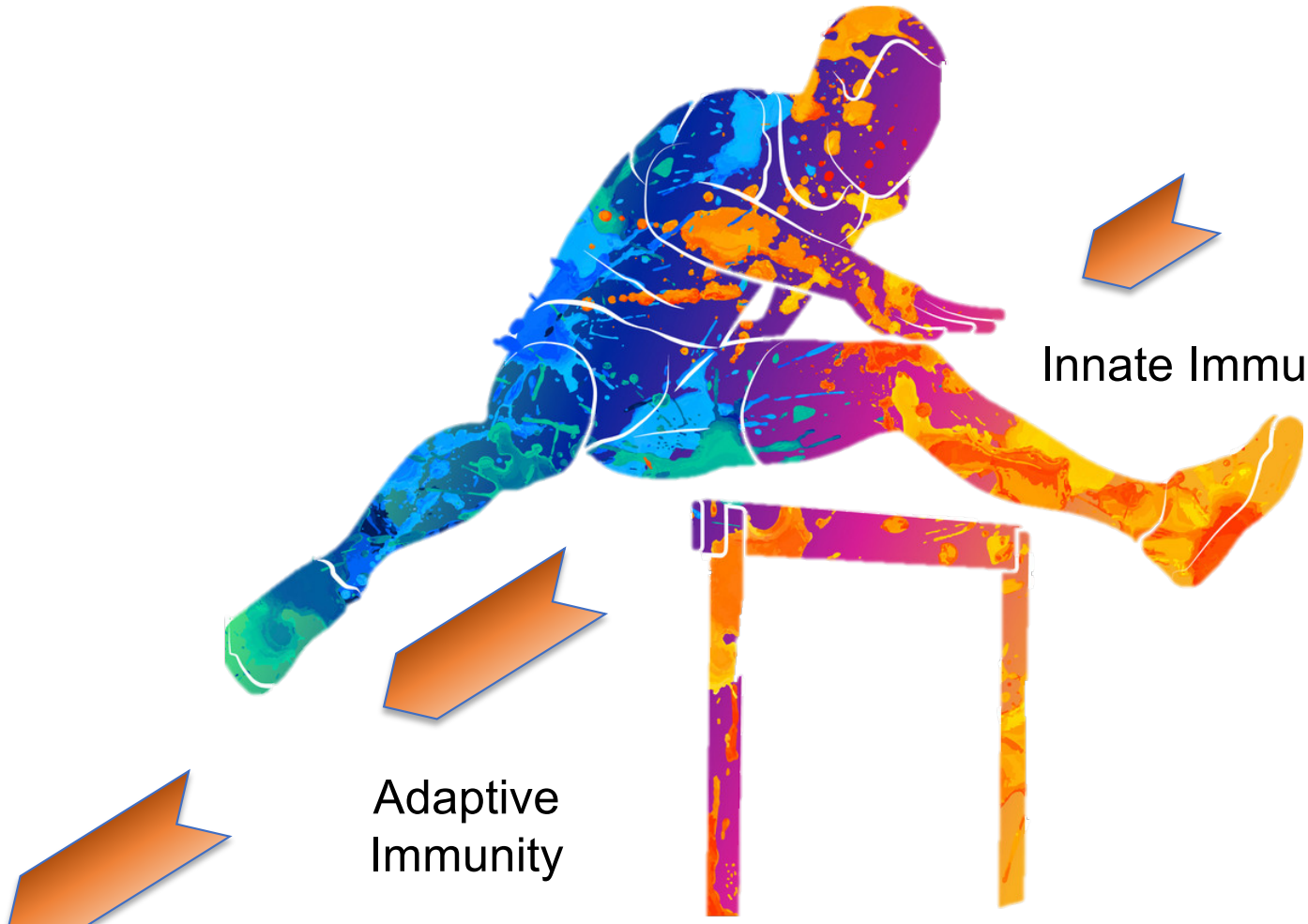
May 2021

Replace and add LA-UR number

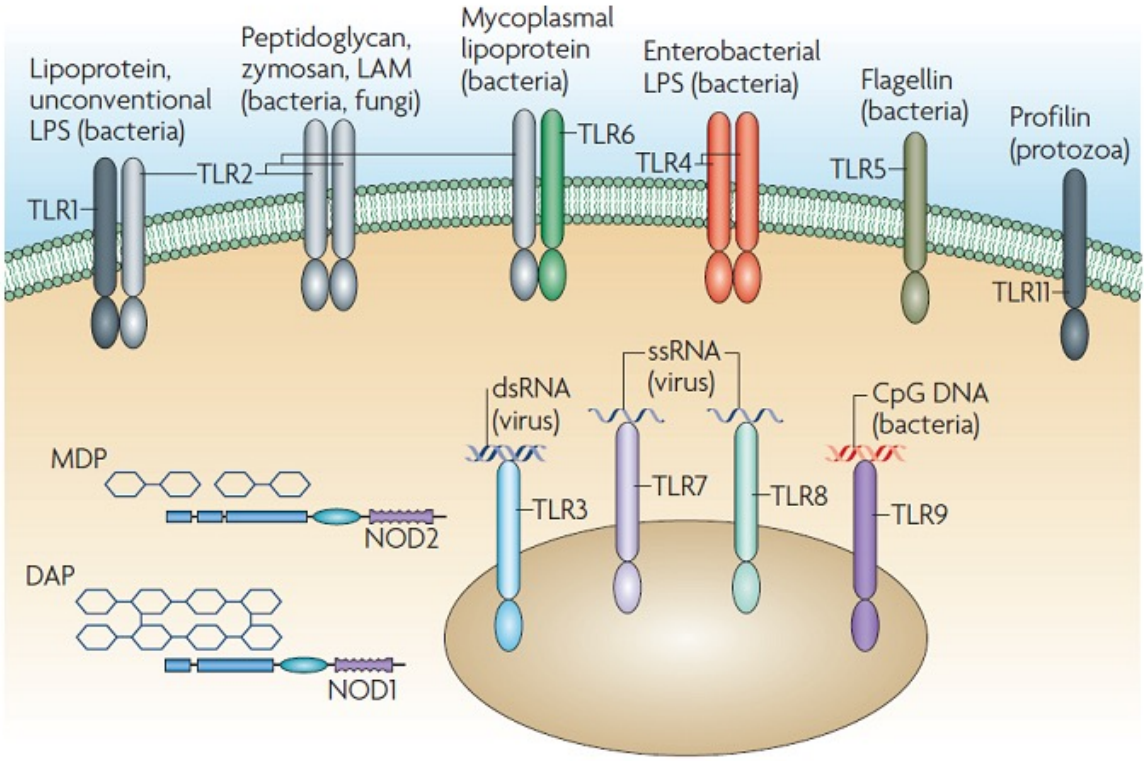


There is a need for universal, agnostic, layered strategies to combat unknown unknowns – especially at the point of need




Physical and chemical
Innate Immunity
Adaptive Immunity

Innate Immunity



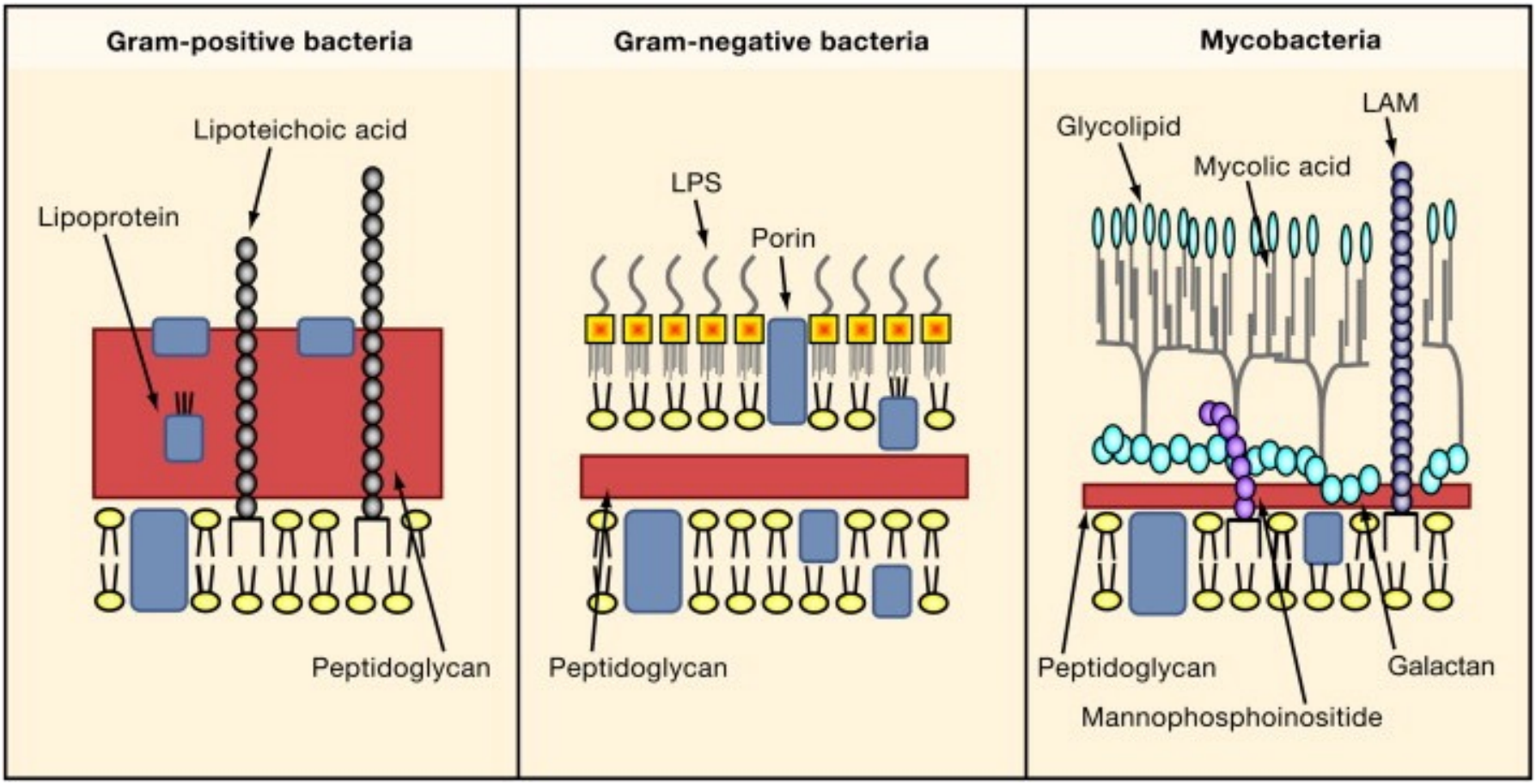
Elegant pattern recognition network

Early (pre-symptomatic) recognition system

Universal

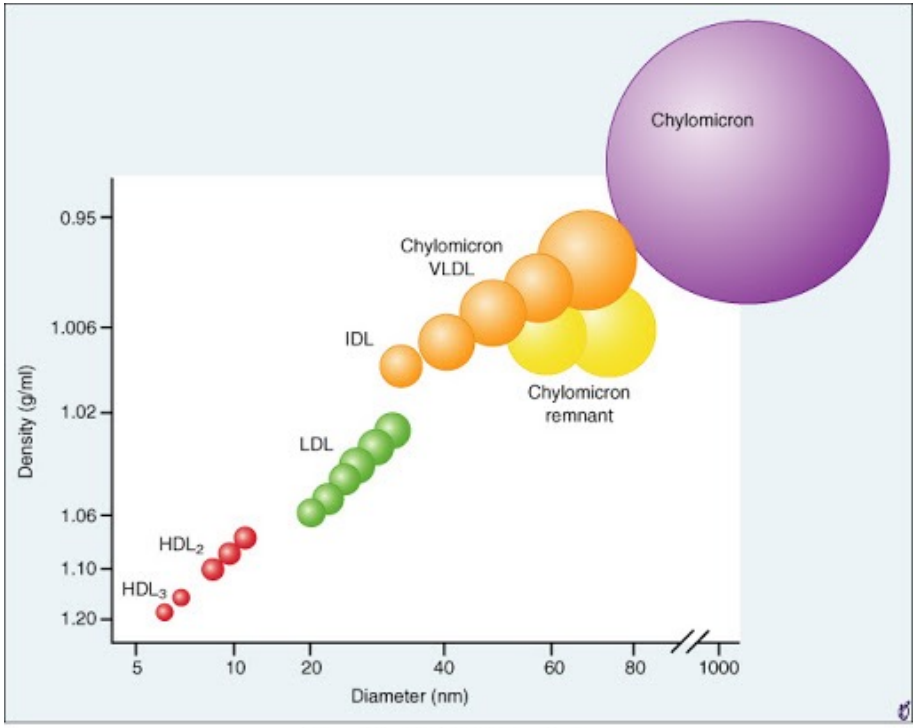
Rapid

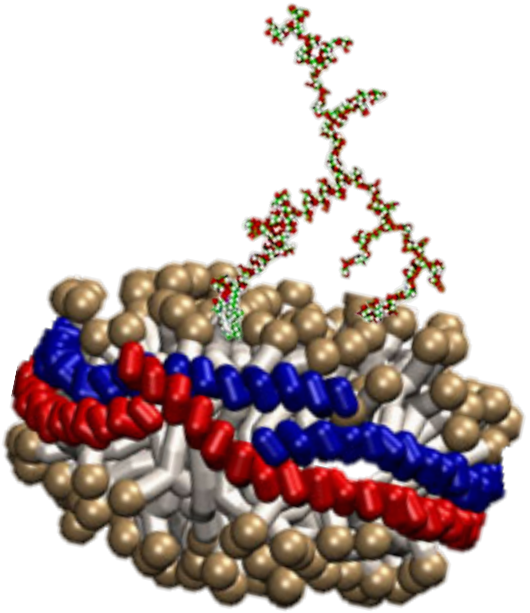
Bacterial Pathogen Associated Molecular Patterns (PAMPs)



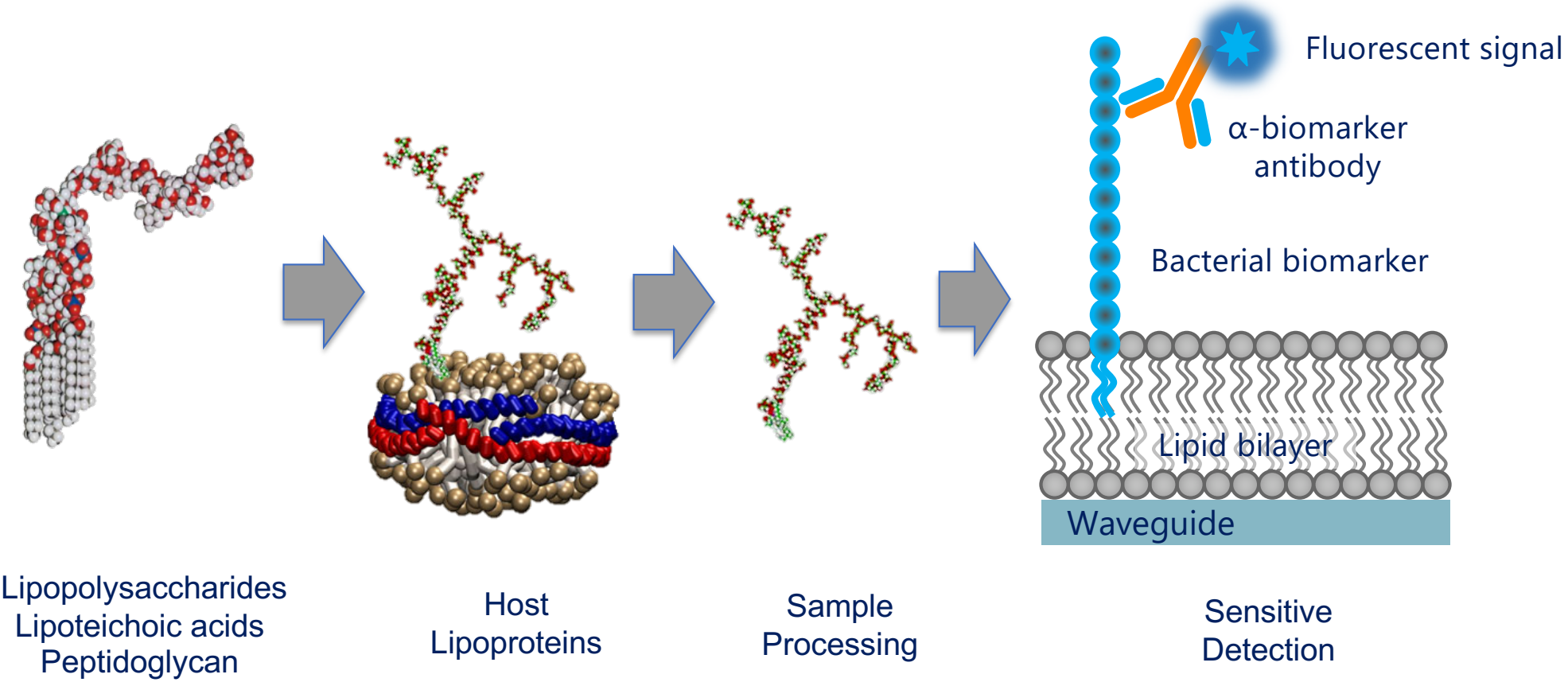
Association of Bacterial Amphiphiles with Host Carriers

Amphiphilic PAMPS universally bind to host lipoprotein carriers (HDL, LDL), and occur associated with them in blood





Universal *Bacterial* Biosensor



Lipopolysaccharides
Lipoteichoic acids
Peptidoglycan
lipoarabinomannans

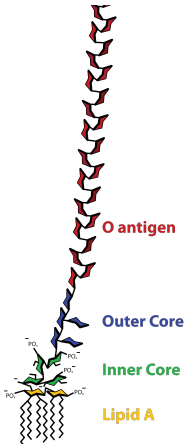
Host Lipoproteins

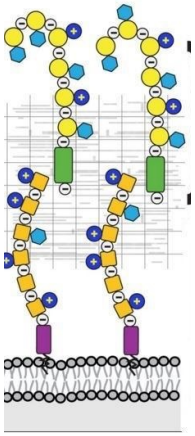
Sample Processing

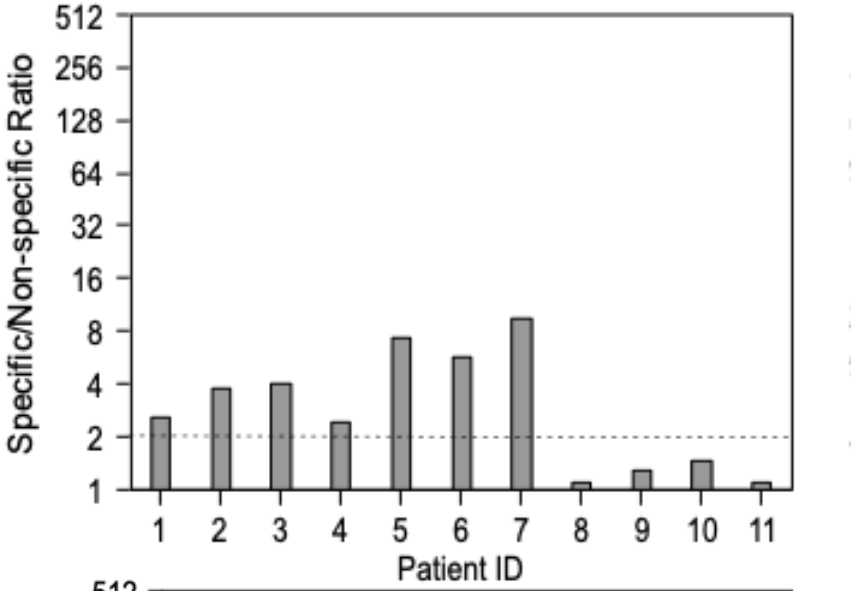
Sensitive Detection

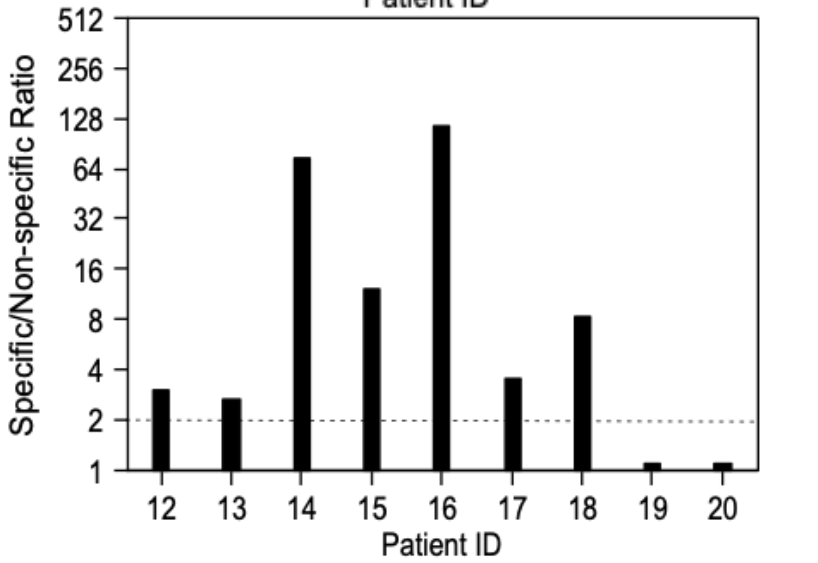
Clinical Validation

Lipopolysaccharides (Gram-negative) and Lipoteichoic acids (Gram-positive) bacterial pathogens



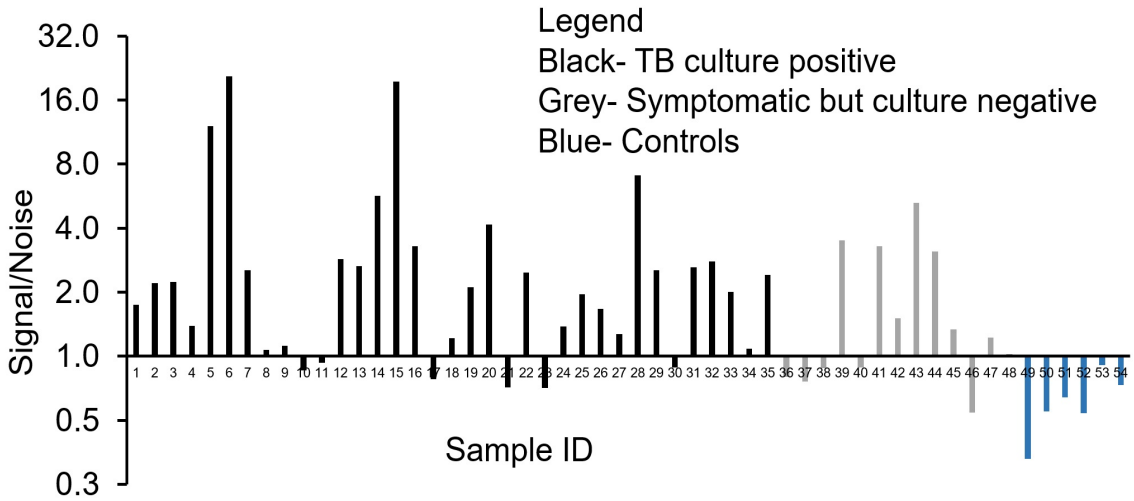


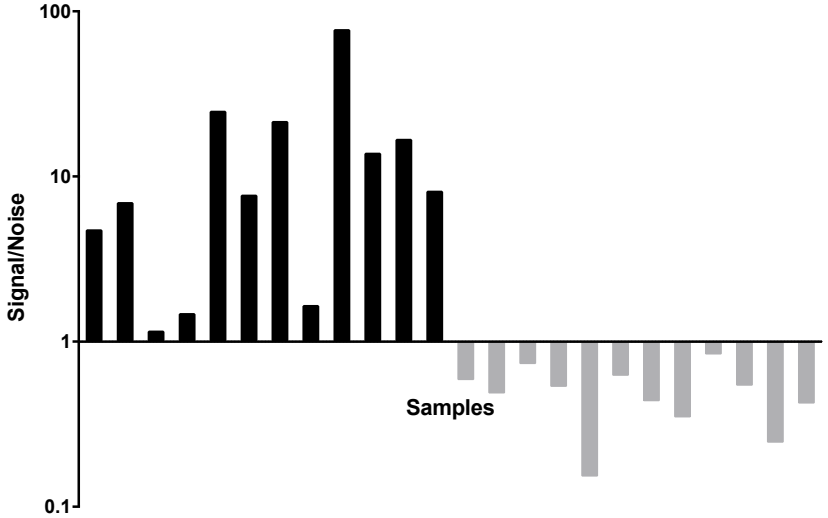


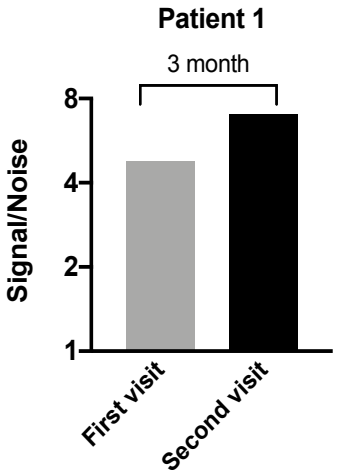


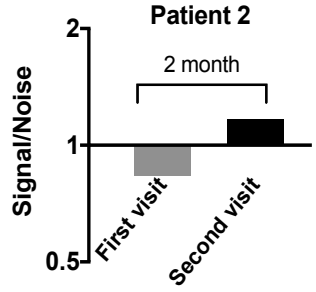
Clinical Validation

Lipoarabinomannans (Gram-indeterminate)



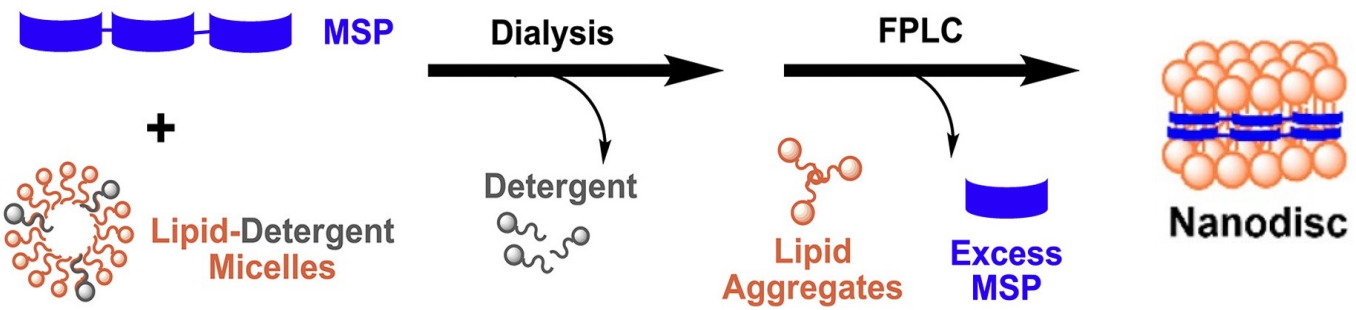


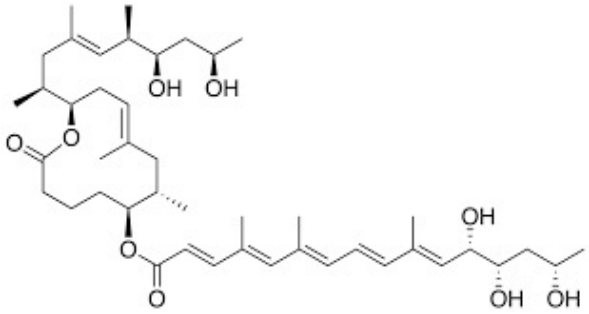




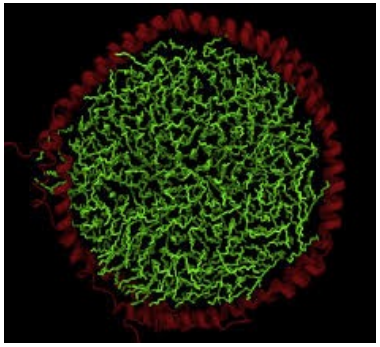
Engineering Lipoprotein Nanodiscs

Reproducible, Controlled Lipoprotein Nanodiscs can be potential ligands for lipids, concentration matrices or early therapeutics





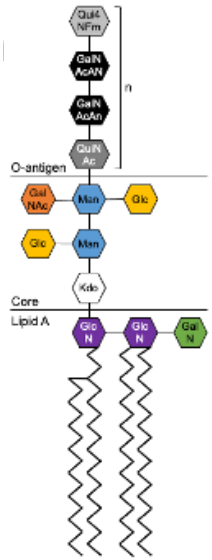
Mycolactone, Small Molecule Toxin associated with Buruli Ulcer



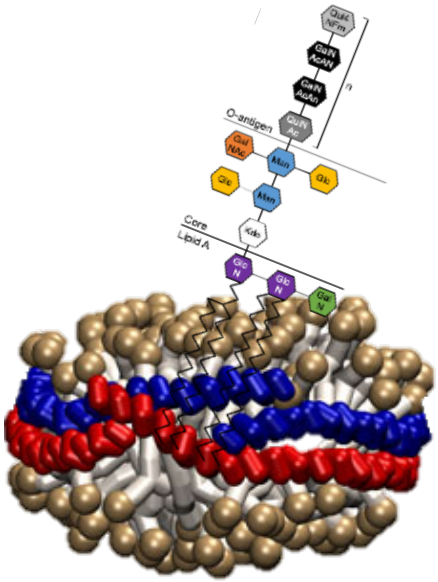
MSP	Lipid	Size (nm)	Zeta (mV)*
1D1	100% DMPC	9.9 ± 2.5	-3.9
	20% DMTAP	10.9 ± 2.8	4.2
	30% DMTAP	8.6 ± 1.5 Multiple peaks	7.9
	40% DMTAP	Multiple large (>100) peaks	N/A
	50% DMTAP	1.591 ± 0.33	N/A

MD simulations and all-atomistic models to generate ND

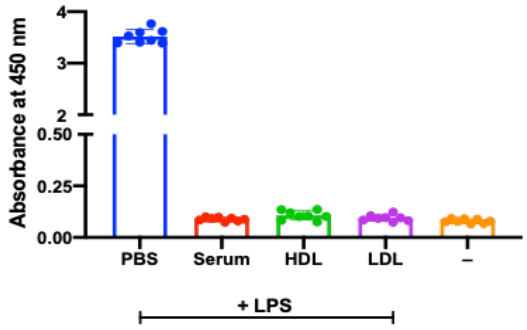
Lipopolysaccharides from *F. tularensis*



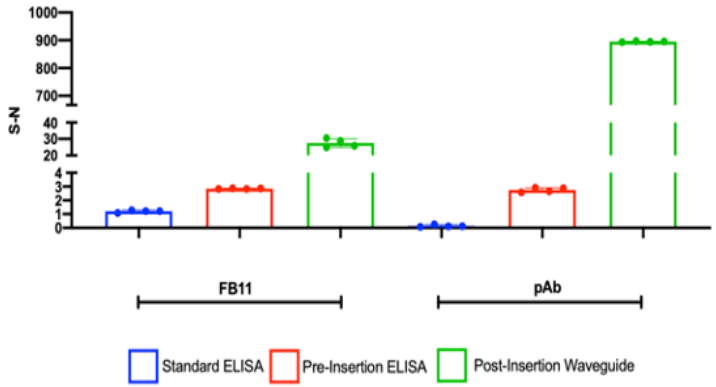
Lipopolysaccharides



Host
Lipoproteins



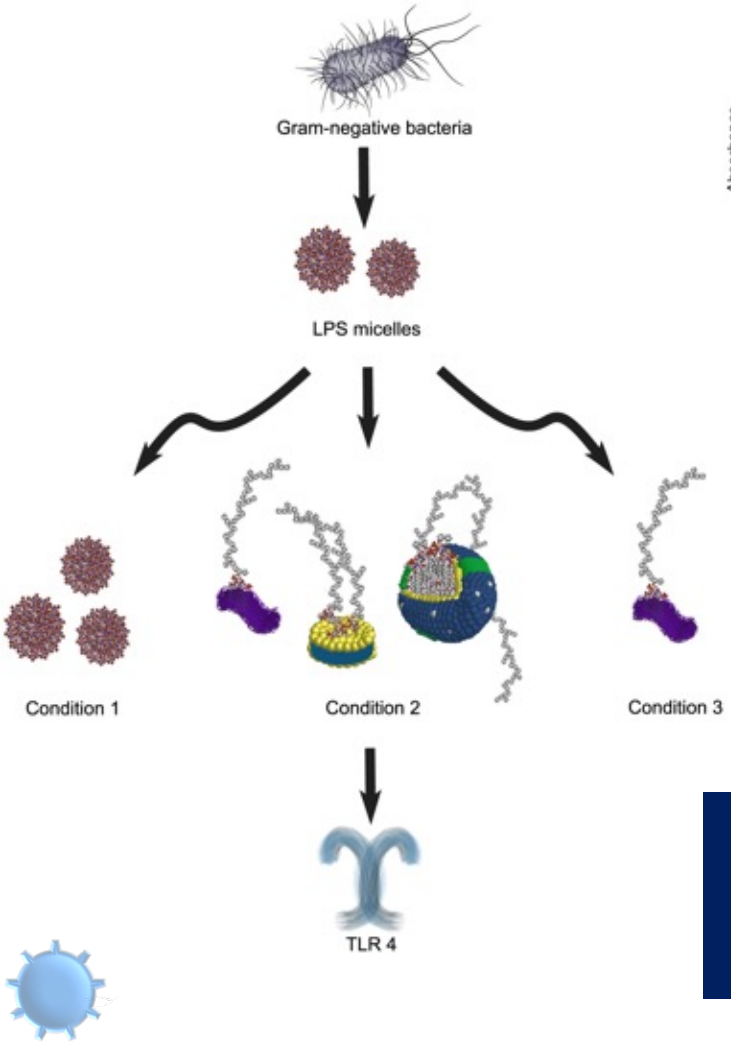
LPS binds to host lipoproteins

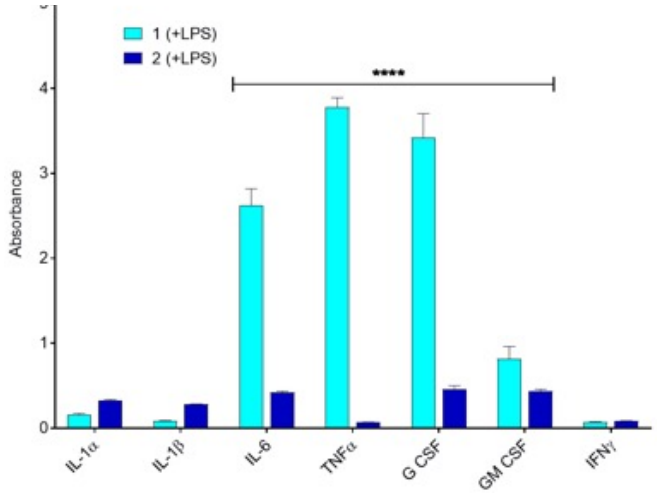


Presentation of LPS impacts assay performance and antibody selection

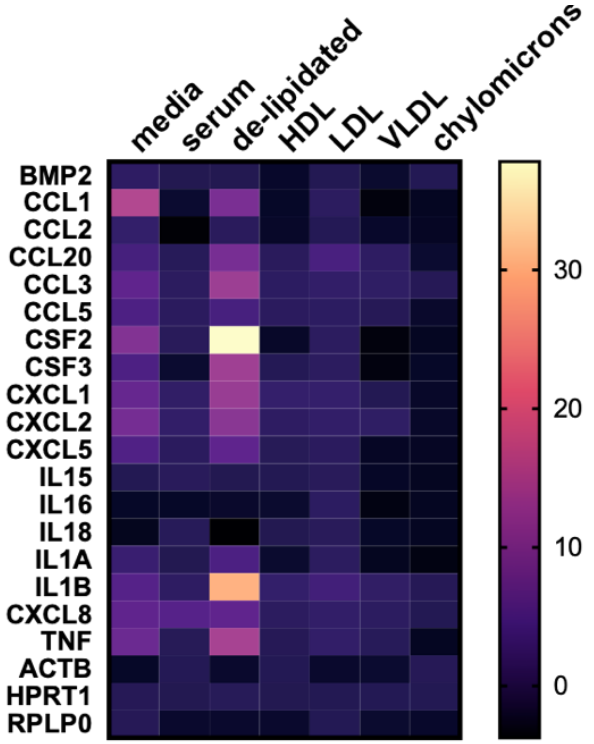
Select agent PAMPs associate with host lipoprotein, and this presentation impacts assay performance

Presentation Matters





Condition 1: LPS in buffer
Condition 2: LPS in serum

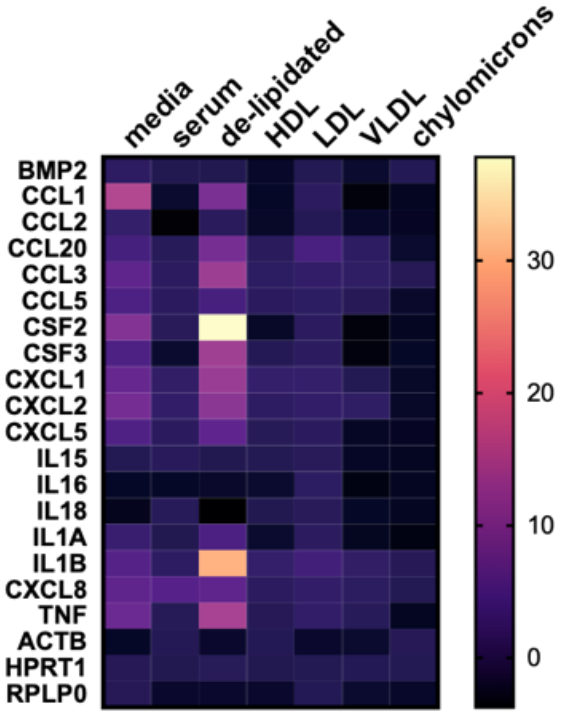


Lipopolysaccharides ***Lipoarabinomannans***

Association with carriers completely changes cytokine profiles – specifically impacting pro-inflammatory cytokines and chemokines

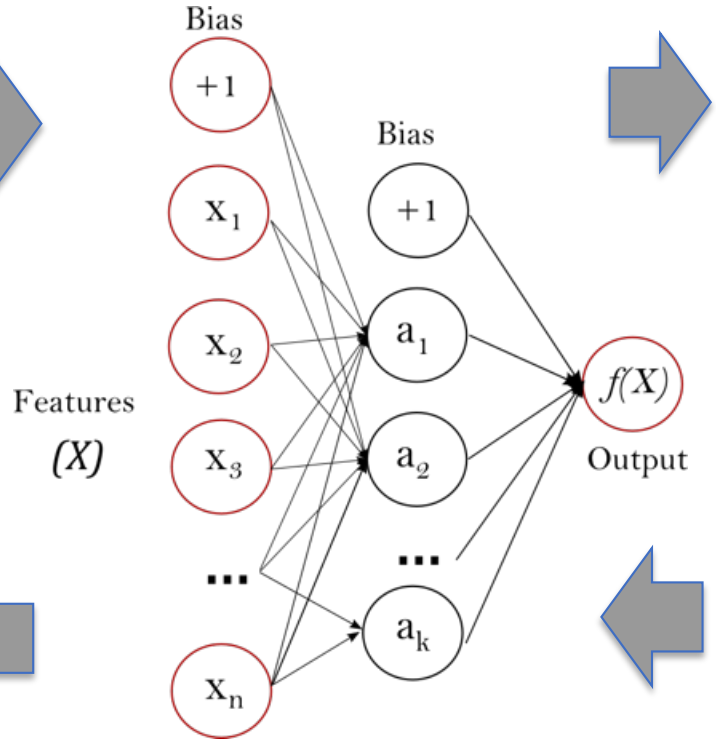
Intelligent Immunity

Laboratory Data



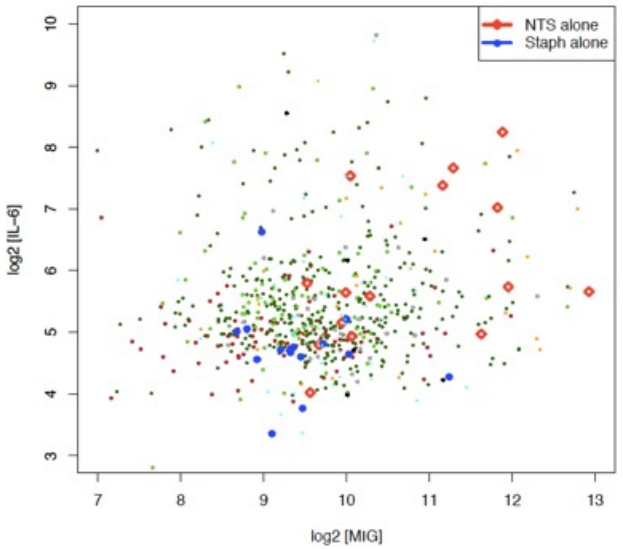
20,000 data points/biomarker combination every 2 weeks

Model Development



Model Development

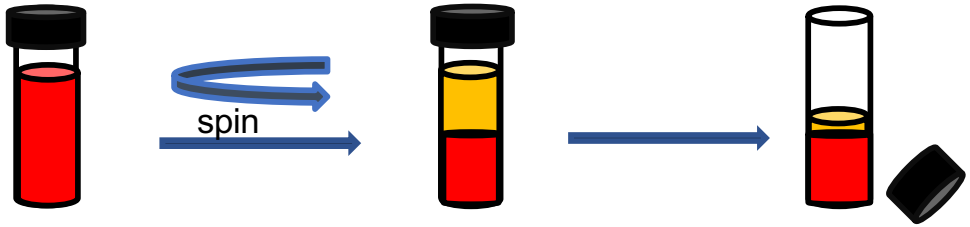
Clinical Validation

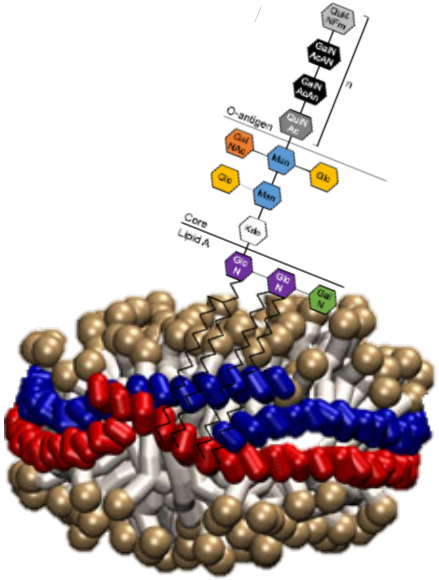


3 time pts/patient, 2200 patients, 18 years, 1600 parameters/collection
= 18,625,200 data points

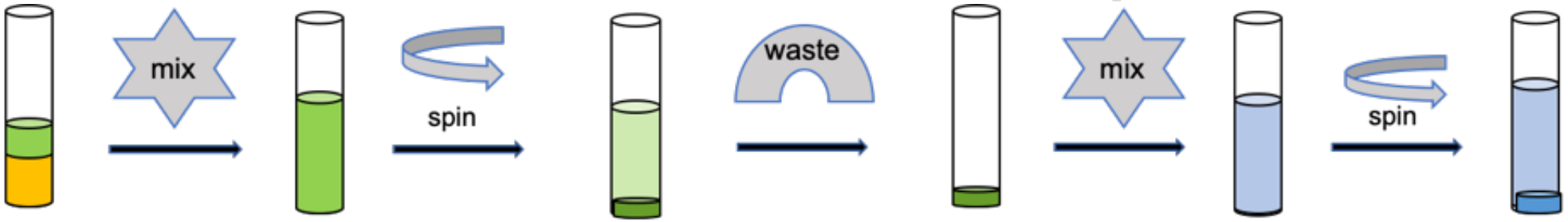
Separation of Proteins and lipids from Blood – The Traditional Way

Step 1. Separation of serum from blood



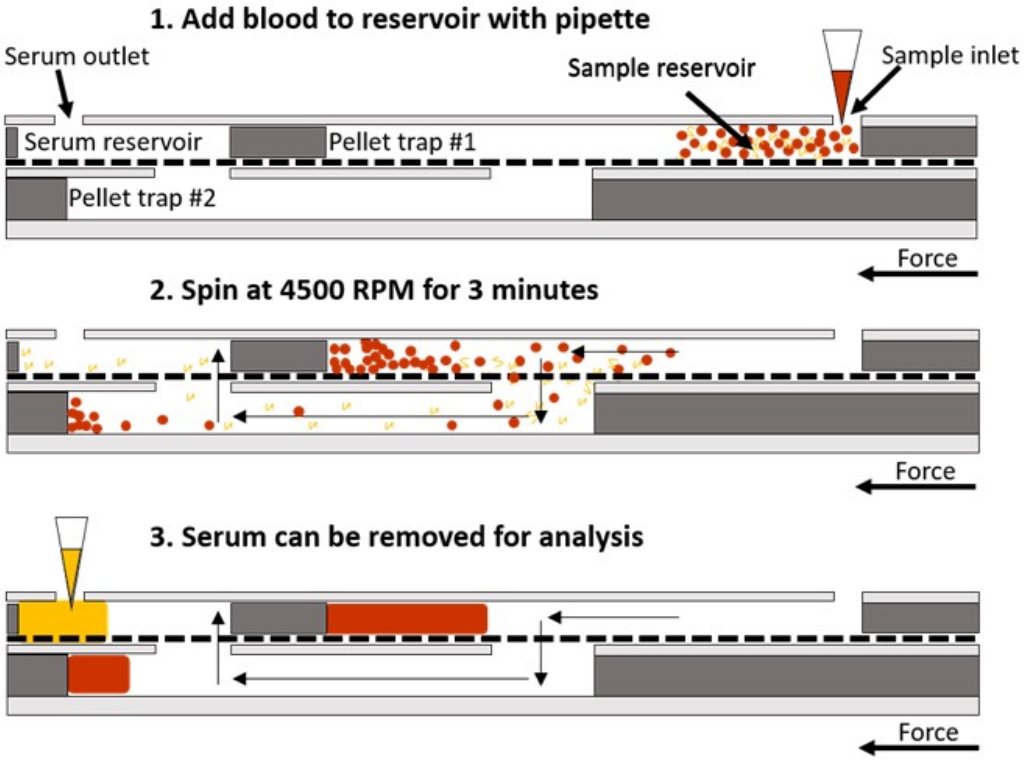


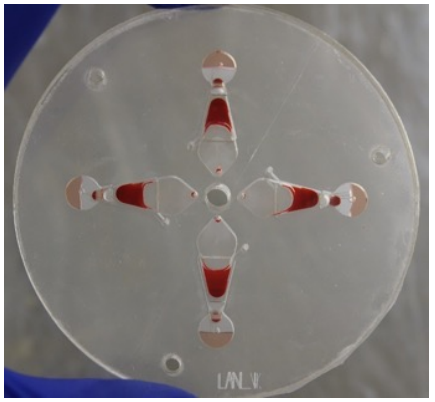
Step 2: Separation of amphiphilic biomarkers

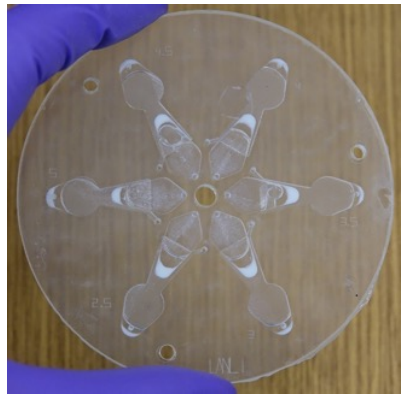


Catch-All, Integrated Sample Processing at the point of need

- Two-tiered novel cross-flow filtration system for separation of aqueous and organic components
- Membrane interfaces compatible with lipid extraction – use of chloroform and methanol in a microfluidics cassette

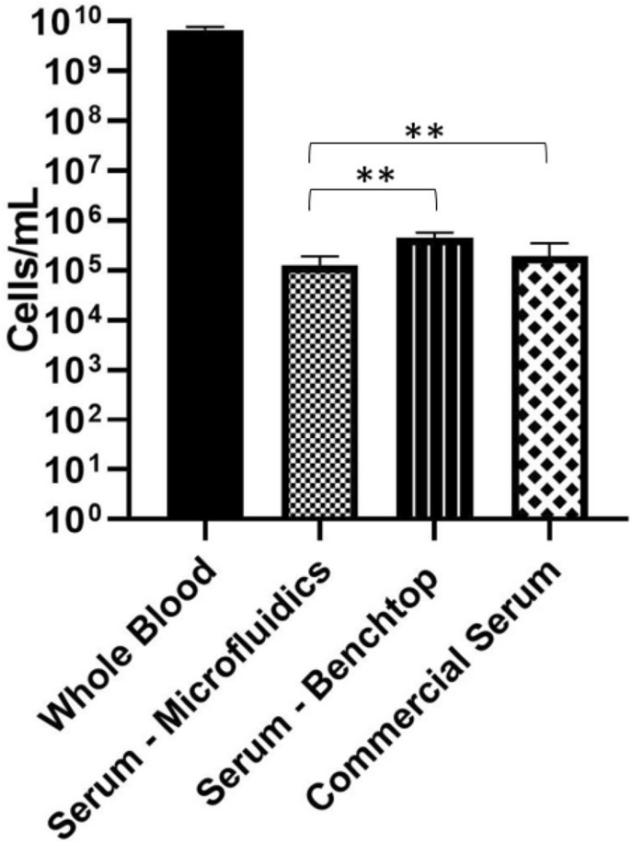


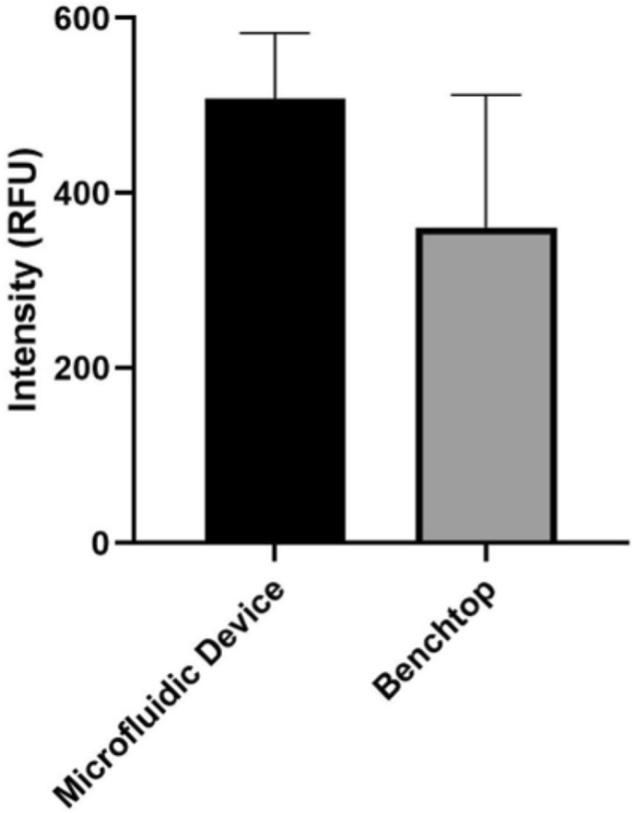




First microfluidic system for separation of lipids and proteins

Performance of the Chip is Comparable to the Laboratory Method

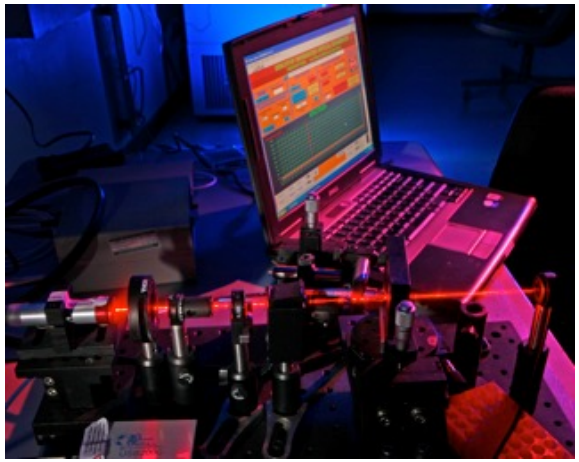




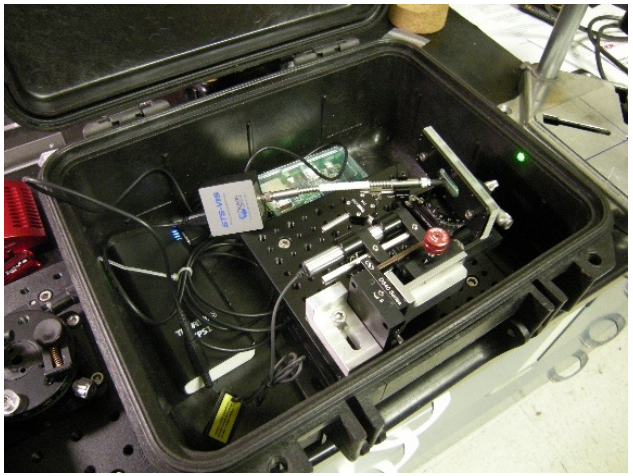
Comparison of Cell Counts (Cells/mL)

Comparison of Lipoarabinomannan Extraction (nM)

Field Ready Waveguide-based Optical Biosensor Platform

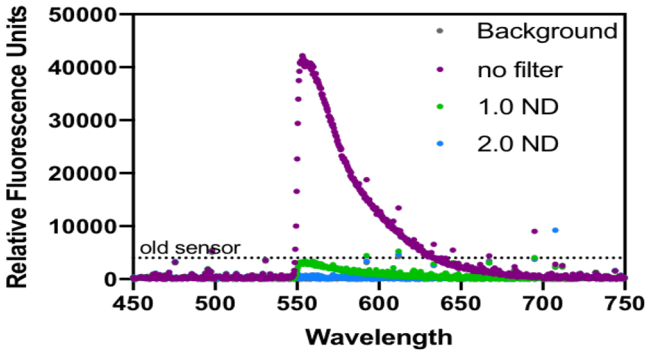


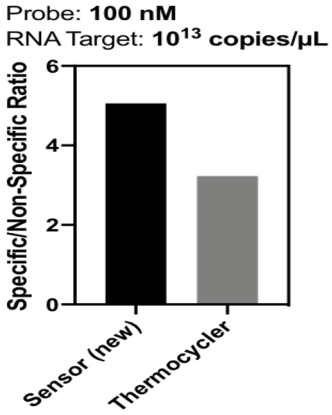
Bench-top Instrument



Field-Ready Instrument

App-based read-out on phone





Comparison of Performance of the Old Vs. New Sensor

- Enhanced dynamic range
- Better sensitivity
- Adapted for amplification-free nucleic acid detection

Universal Bacterial Biosensor

Harshini Munkundan and team

- ***Comprehensive:*** *Identifies any bacteria from a small sample of water or blood*
- ***Robust:*** *Cannot be tricked by bacterial evolution or engineering*
- ***Fast:*** *Runs in 30 minutes from sample collection to result*
- ***Easy:*** *Can be performed by a non-expert at the point of need with no laboratory equipment*
- ***Flexible:*** *Interfaces with other detection systems, can be adapted to detect other oily biomarkers for diseases like cancer*

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2018 R&D 100 WINNER

2021 R&D 100 ENTRY

PEGASUS

Portable EnGineered Analytic Sensor with aUtomated Sampling

Novel analytical tool brings the lab to your fingertips

Innovative: Supplies point-of-need biochemical analysis

Rapid: Delivers accurate results in less than 30 minutes

Versatile: Detects a variety of biomarkers and biochemicals

Unique: Processes samples with a centrifugal microfluidic device

Robust: Provides a rugged, portable unit to resource-poor settings

Los Alamos NATIONAL LABORATORY

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BIOLOGY	CHEMISTRY	ENGINEERING	THEORY	COLLABORATORS
Jessica Kubicek-Sutherland	***Laura Lilley***	***Kiersten Lenz***	***Sara DelValle***	University of New Mexico
Loreen Stromberg	Aaron Anderson	Aaron Anderson	Nick Hengartner	National Institutes of Allergy and Infectious Disease
Zachary Stromberg	Joanna Casson	Jennifer Harris	Ben McMahon	Johns Hopkins University
Alexis Bitzer	Stosh Kozimor	David Mascarenas	Carrie Manore	Univ Nebraska Medical Center
Shailja Jakhar		John Morales		Foundation for Innovative New Diagnostics
				Medical University of South Carolina

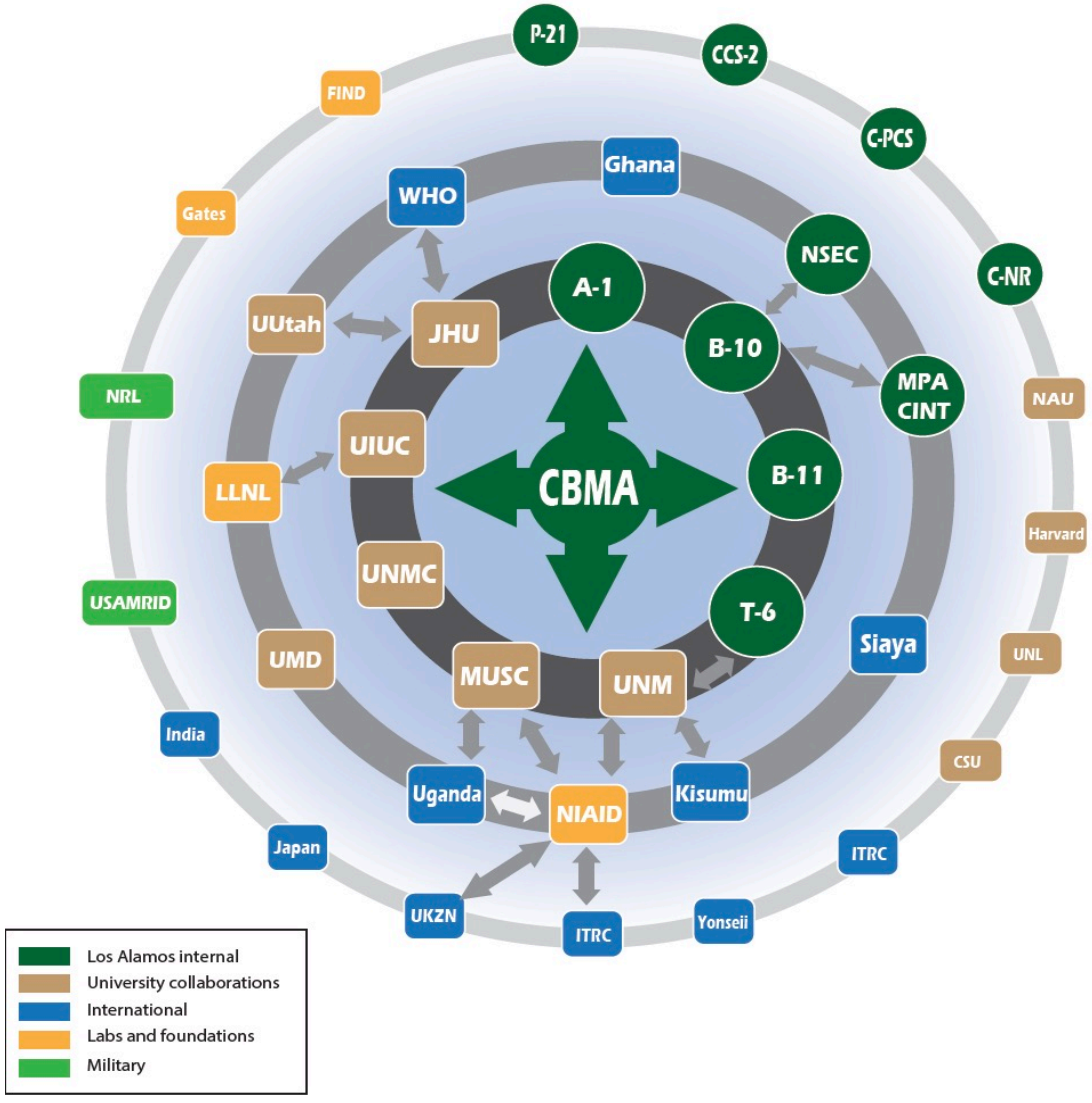
FIND Because diagnosis matters

LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT WHERE INNOVATION BEGINS

DEFENSE THREAT REDUCTION AGENCY UNITED STATES OF AMERICA

NIAID

USDA



Nature's response to infectious disease is agnostic

Why isn't ours?